REMARKS

Claims 7-10, 12, 14, 23, and 28-42 are now pending in the present application, new claims 28-42 having been added by the above amendment. Support for new claims 28-34 can be found in Example 16 at page 34. Support for new claims 35-42 can be found in claims 12 and 14 as originally filed.

Applicant has discovered a method for producing a particular optically active compound, (S)-halo-3-hydroxybutyric acid ester, in a high degree of optical purity. The method utilizes a purified acetoacetyl-CoA reductase enzyme to convert a 4-halo-acetoacetic acid ester or its derivative into the desired optically active product. One such product, ethyl (S)-4-chloro-3-hydroxybutyrate, referred to herein as "SECHB", is a starting material for producing Lipitor® (atorvastatin calcium), one of the best selling drugs in the world. As the optical purity of the SECHB starting material is critical, methods such as those presently claimed are extremely valuable.

Claims 7-10, 12, 14, and 23 stand rejected under 35 U.S.C. §103(a) as allegedly unpatentable over Matsuyama *et al.* (U.S. Patent No. 5,559,030) in view of Peoples *et al.* (U.S. Patent No. 5,229,279) or Somerville *et al.* (WO 93/02187). This is the only ground for rejection cited.

The position taken by the Patent Office is that Matsuyama *et al.* teach a process for the production of optically active 4-halo-3-hydroxybutyric acid ester (either the (S) or the (R) form) by contacting 4-halo-3-acetoacetic acid ester with a microorganism or a preparation thereof, and that one of ordinary skill in the art would have been motivated to substitute the cloned acetoacetyl-CoA reductases disclosed by Peoples *et al.* and Somerville *et al.* in the method of producing (S)-4-halo-3-hydroxybutyric acid ester disclosed by Matsuyama *et al.*

Applicant respectfully submits that the Patent Office has failed to make out the requisite *prima facie* case that the presently claimed invention is obvious. As discussed in detail below, one of ordinary skill in the art would find in the cited references neither motivation to combine the references nor a reasonable expectation of success in achieving Applicant's claimed invention. Each of these elements can be found only in Applicant's own disclosure.

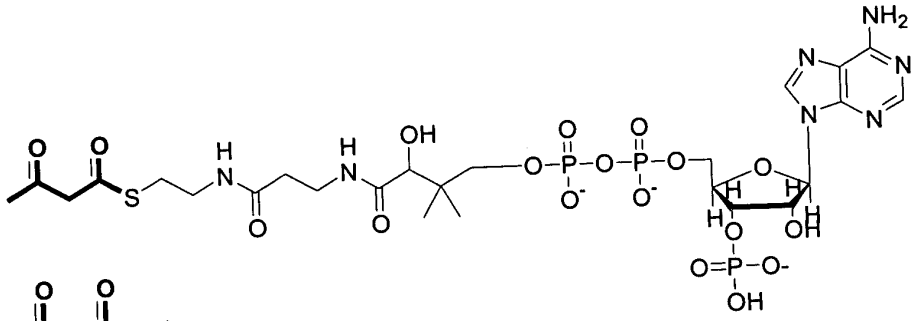
Furthermore, Applicant's invention produces results that are quite unexpected in view of what is known in the art.

Matsuyama *et al.* disclose the use of cell cultures and preparations thereof (e.g., lysed or immobilized cells – see col. 6, lines 1-19) in an asymmetric reduction reaction that produces, from 4-halo-acetoacetic acid ester substrates, various optically active 4-halo-3-hydroxybutyric acid esters (of both S and R configurations), including SECHB. Numerous microorganisms that can be used for this purpose are named; however, neither *Ralstonia* species (previously known as *Alcaligenes*), specified in present claims 8 and 9, nor *Zooglooea* species, specified in present claim 23, is among them. As the Examiner will agree, the whole organisms and lysed cell contents used in the Matsuyama *et al.* reduction of course comprise complex metabolic pathways and a milieu of enzymes and co-factors. Matsuyama *et al.* fail to identify any specific enzyme (acetoacetyl-CoA reductase or any other) or even an enzymatic pathway involved in the reduction reaction. Indeed, the Examiner has acknowledged that "Matsuyama does not teach the enzyme. . ." (See, e.g., the Final Office Action dated March 12, 2002, at page 3, lines 18-19.)

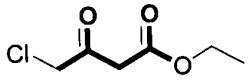
To address this acknowledged deficiency of Matsuyama *et al.*, the Examiner cites two secondary references: Peoples *et al.* and Somerville *et al.* Each of these publications describes methods for producing a polymer, poly-beta-hydroxybutyric acid, using acetoacetyl-CoA reductase to carry out one step (converting acetoacetyl-CoA to D(-)-3-hydroxybutyryl-CoA) of the polymer's three-step synthesis. The processes described by these two secondary references thus differ starkly from the presently claimed process in that they utilize a different substrate and arrive at a different product. Neither of these two references teaches or suggests use of the substrate specified in the claims, or production of the product specified by the claims. In fact, it appears that the only nexus between the presently claimed methods and the methods disclosed in Peoples *et al.* and Somerville *et al.* is the use of the enzyme acetoacetyl-CoA reductase.

The structural differences between (1) the acetoacetyl-CoA substrate utilized by Peoples *et al.* and Somerville *et al.*, and (2) the halogenated, non-CoA-containing substrate specified in the claims, are illustrated below. The acetoacetyl backbone common to both compounds appears in bold.

AA-CoA



ECAA



According to the Examiner, it would have been obvious to design a method for producing (S)-4-halo-3-hydroxybutyric acid ester comprising asymmetrically reducing 4-halo-acetoacetic acid ester or its derivative as taught by Matsuyama *et al.*, but using the acetoacetyl-CoA reductase constituting the poly-β-hydroxy fatty acid biosynthesis system from *Alcaligene eutrophus (Ralstonia eutropha)* as taught by People *et al.* or Somerville *et al.* As set forth in the Office Action dated March 12, 2002, as well as previous Office Actions in this case, the supposed motivation for using the acetoacetyl-CoA reductases of Peoples *et al.* or Somerville *et al.* in Matsuyama *et al.*'s conversion reaction is that the nucleic acids encoding these enzymes have been cloned and therefore this allows for more control in the reaction conditions and the opportunity to improve the enzyme by the use of recombinant technologies and mutagenesis. A further motivation is postulated on page 3 of the March 12, 2002, Office Action: that each of Peoples *et al.*, Somerville *et al.* and Matsuyama *et al.* "involves the reduction of acetoacetic acid or its derivative to hydroxybutyrate or its derivative using acetoacetyl CoA reductase." The Examiner appears to recognize that this breathtakingly conclusory characterization of Matsuyama *et al.* is unsupported by anything in Matsuyama *et al.*, as he goes on to acknowledge that "Matsuyama *et al.* does not teach the enzyme responsible for such a conversion." This lack of support in Matsuyama *et al.* does not trouble the Examiner, however. According to the Examiner, "one of ordinary skill in the art, given the teachings of Peoples *et al.* or Summerville (*sic*) *et al.*, who teach a similar conversion, would expect that the same enzyme would be responsible for such a conversion irregardless of the presence of an extraneous halogen group." No evidence is provided for this "expectation". Furthermore, it ignores the fact that the "extraneous" halogen group on Matsuyama *et al.*'s substrate is not the only difference, or even

the most striking difference, between the substrates. Applicant directs the Examiner's attention to the seemingly-overlooked fact that the substrate of Matsuyama *et al.*, and of the present claims, has an ethyl ester instead of the CoA thioester present on the substrates utilized by Peoples *et al.* and Somerville *et al.*

It appears that the Examiner, presumably unable to find evidence in the prior art that would bridge the gap between the primary reference and the secondary references, believes his unsupported opinion can substitute for such evidence. The reader is asked to take the Examiner's word that one of ordinary skill, reading the cited references, would have at least six insights not found in the prior art:

FIRST, this person of ordinary skill would somehow just "know" that the acetoacetyl-CoA reductase can act on a substrate that does not contain the CoA moiety, even though the disclosures of Peoples *et al.* and Somerville *et al.* were limited to use of a CoA-linked substrate, and even though the enzyme's very name indicates that it is specific for a CoA-containing substrate;

SECOND, the person would also just "know" that the enzyme can utilize a halogenated substrate, even though the disclosures of Peoples *et al.* and Somerville *et al.* were limited to non-halogenated substrates, and no other evidence of wider activity of acetoacetyl CoA reductase is to be found in the art;

THIRD, the person would realize, without any indication in the prior art, that this halogenated, non-CoA-containing substrate would produce an optically active product when acted upon by the enzyme;

FOURTH, the person would have guessed that the product would be the (S) stereoisomer, even though Matsuyama *et al.* demonstrates that some microorganisms instead produce the (R) stereoisomer from the same substrate;

FIFTH, the person would have divined that acetoacetyl CoA reductase was therefore the one and only enzyme utilized in the reactions disclosed by Matsuyama *et al.*, even though there is no evidence that the microorganisms utilized by Matsuyama *et al.* even express this enzyme and even though other families of enzymes are capable of carrying out the reaction (see below); and

SIXTH, the person would have concluded that one should therefore substitute purified acetoactyl CoA reductase for the whole and/or lysed cells of Matsuyama *et al.*

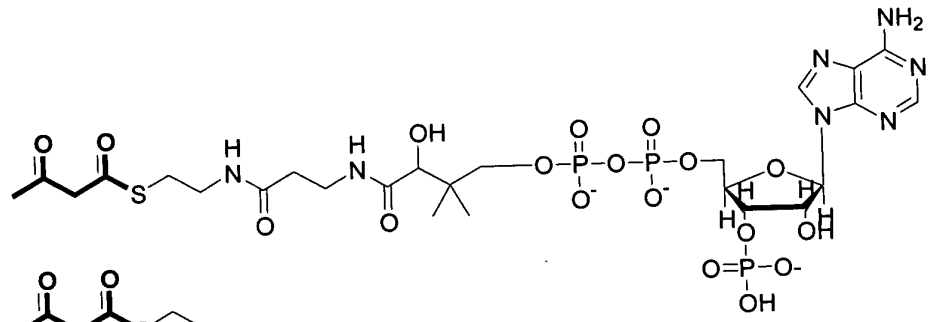
Applicant fails to see how anyone could draw any of these conclusions, let alone all of them, without using the present application as a roadmap. Yet every one of these insights would be necessary to make the leap from the cited art to the claimed invention. The Examiner has simply failed to make the case that the prior art, rather than Applicant's own disclosure, provided the requisite motivation and expectation of success to make the present invention. As the Examiner has not met his burden, the rejection should be withdrawn.

Although the above arguments are more than sufficient to rebut the Examiner's *prima facie* case, Applicant submits below further evidence in support of the nonobviousness of the invention.

For example, when Fukui *et al.* (Biochim. Biophys. Acta. 917:365-371, 1987; Appendix A) studied the substrate specificity of purified acetoacetyl-CoA reductase derived from *Zoogloea reamigera*, they looked at 14 different potential substrates, every one of which contained CoA, and not one of which was halogenated (see Table II on page 369). There is no suggestion in Fukui *et al.* that this enzyme could act on non-CoA-containing substrates or on halogenated substrates; in fact, the implication is to the contrary since none were even tested. Moreover, Fukui *et al.* found that the enzyme is highly substrate-specific: of the 14 CoA-containing compounds tested, only two turned out to serve as substrates at all, and one of those two (a five-carbon substrate) showed only 4.8% the activity of the other (a four-carbon substrate) (see Table II). One reading this would understand that the enzyme has an extremely limited range of available substrates, so that a radically different compound, such as one that (a) is halogenated, (b) is not a thioester and (c) lacks CoA, is unlikely to work.

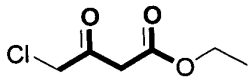
The following table summarizes certain non-prior art evidence showing that the ability of various reductases to utilize three different substrates differs markedly from enzyme to enzyme, and so cannot be predicted *a priori*. The three substrates tested are illustrated below with their common acetoacetyl backbone shown in bold. Note that the middle one, EAA, has an ethyl ester like that of ECAA instead of the CoA thioester of AA-CoA, but like AA-CoA lacks the chloro moiety of ECAA. EAA could therefore be said to represent a structure midway between ECAA and AA-CoA.

AA-CoA



EAA

ECAA



Enzyme	Substrate Specificity		
	AA-CoA	EAA	ECAA
ZrAR1	100%	14%	35%
ReAR1	39%	1%	100%
EcKR1	100%	2%	86%
BsKR1	22%	5%	100%
SvKR1	100%	0.2%	0

ZrAR1: Acetoacetyl-CoA reductase from *Zoogloea ramigera* (data derived from Table 2 of Declaration of Hiroaki Yamamoto under 37 C.F.R. §1.132 filed August 11, 2000).

ReAR1: Acetoacetyl-CoA reductase from *Ralstonia eutropha* (data derived from Second Declaration of Hiroaki Yamamoto under 37 C.F.R. §1.132, filed herewith).

EcKR1: ß-ketoacyl-ACP reductase from *Escherichia coli* (data derived from the specification at pages 21-26, see particularly Table 1 at page 26; the data are presented relative to 100% activity with AA-CoA).

BsKR1: ß-ketoacyl-ACP reductase from *Bacillus subtilis* (data derived from Second Declaration of Hiroaki Yamamoto under 37 C.F.R. §1.132, filed herewith).

SvKR1: Ketoacyl reductase from *Streptomyces violaceoruber* (data derived from Second Declaration of Hiroaki Yamamoto under 37 C.F.R. §1.132, filed herewith).

It can be seen from the above table that, contrary to the assumptions underlying the obviousness rejection, one cannot predict an enzyme's ability to use a halogenated, non-CoA-containing substrate based on its activity with respect to a non-halogenated, CoA-containing substrate. Though EAA has a structure midway between that of AA-CoA and ECAA, it is in general utilized at a much lower rate than either AA-CoA or ECAA. Thus, the absence of the CoA thioester has a significant effect on activity, one that is inexplicably made up for (and in some cases overcompensated) by the addition of a chloro moiety at the opposite end of the molecule. Applicant fails to see how this could have been predicted. Furthermore, in direct contrast with what the Examiner says one of ordinary skill would "expect", one enzyme (SvKR1) that was able to utilize AA-CoA showed no activity at all with ECAA, and almost no activity with EAA. These data illustrate that one could not have predicted the effects of changes to the substituents on the AA-CoA substrate of Peoples *et al.* and Somerville *et al.* It was not at all a given that acetoacetyl-CoA reductase could utilize substrates different than those described in Peoples *et al.* and Somerville *et al.* It is even less of a given that this enzyme would produce predominantly the S-enantiomer, as opposed to the R-enantiomer or a racemic mixture. The Patent Office has provided no evidence beyond unsupported speculation that one of ordinary skill would have "expected" this activity of an enzyme never before shown to possess it.

Yet another unsupported speculation is the Examiner's assertion that, because Peoples *et al.* and Somerville *et al.* teach that acetoacetyl-CoA reductase can convert AA-CoA to

hydroxybutyrate, one of ordinary skill would expect the same enzyme to be precisely the one that accounts for Matsuyama *et al.'s* observed reactions. Even if there were some indication in the art that acetoacetyl-CoA reductase could utilize the halogenated, non-CoA-containing substrate of Matsuyama *et al.* (and there isn't), there is no basis in any of the cited art for assuming that this particular enzyme in fact accounted for any of Matsuyama *et al.*'s observed reactions (much less for the reactions that produced the S-enantiomer specifically). For example, any or all of the reactions in Matsuyama *et al.* might have been catalyzed by a fatty acid synthase present in the various microorganisms. Indeed, this class of enzymes, unlike the acetoacetyl-CoA reductase specified in the claims, was well known in the art to be able to catalyze the conversion of 4-halo-acetoacetic acid ester to (S)-halo-3-hydroxybutyric acid ester (see specification at pages 1-3). It is puzzling why the Examiner attributes the reaction observed by Matsuyama *et al.* to a previously-unknown activity of acetoacetyl-CoA reductase, rather than to this known activity of fatty acid synthases. Other examples of enzymes that, in retrospect, might account for Matsuyama *et al*'s observations include carbonyl reductases (see, e.g., Wada *et al.*, J. Biosci. Bioeng. 87:144-148, 1999; and Yamamoto *et al.* (Biosci. Biotech. Biochem. 66:1775-1778, 2002; Appendices B and C, respectively), and short chain alcohol dehydrogenase/reductases (Wada *et al., supra*). Furthermore, as illustrated in Wada *et al.*, any given organism may possess several distinct enzymes that catalyze the asymmetric reduction of ECAA. Clearly, one cannot predict from what is disclosed in Matsuyama *et al.* just what enzyme or enzymes produced the results observed with each of the many different types of whole or lysed cells utilized in that reference. The Examiner's attempt to make that prediction is simply not supported by the facts.

Finally, Applicant submits that the present invention produces results that are quite surprising in view of what the prior art would have led one to expect. As the Examiner is aware, evidence of unexpected results is highly probative of the nonobviousness of an invention, and so must be taken into account by the Patent Office.

One example of these surprising results is described above in the discussion of the relative activities of two acetoacetyl-CoA reductases (ZrAR1 and ReAR1) with different substrates (see the Table above). Based on structural similarities, one would have expected that the enzymes would exhibit the following relative specificity for the three tested substrates:

AA-CoA > EAA > ECAA (if they could utilize the latter two at all). Surprisingly, neither enzyme behaved as expected. ZrAR1, (which falls within present claim 23) showed a relative specificity of AA-COA > ECAA > > EAA. ReAR1 (which falls within claims 8-10) exhibits the even more surprising relative ranking of ECAA > AA-CoA > EAA. Nothing that was known in the art about acetoacetyl-CoA reductases could have led one to predict that ECAA would rank above EAA, as it does for both of these enzymes. Quite startling is the observation that for ReAR1, ECAA ranks even above the known substrate, AA-CoA.

Another aspect of the invention that qualifies as unexpected results is the degree of optical purity of the product produced in the claimed method. It can be seen from Matsuyama *et al.* that the optical purity of ECHB produced by whole cells can vary from 37 to 99%e.e. for cells that produce the S-enantiomer and from 49 to 94%e.e. for cells that produce the R-enantiomer. Only six of the 37 different strains tested by Matsuyama *et al.* achieved 99% optical purity. It was thus quite unexpected that the presently claimed method, utilizing an enzyme never previously known to catalyze the conversion of ECAA to ECHB, not only can produce ECHB, but is stereospecific for the highly desired S-enantiomer (as opposed to the R-enantiomer or a racemate), and furthermore produces 99% or higher optical purity. See Example 16 of the specification and Table 3 of the Declaration of Hiroaki Yamamoto under 37 C.F.R. §1.132 filed September 20, 2000.

In summary, there was neither motivation to combine the cited references to produce the claimed invention, nor a reasonable expectation of success even if one were to combine them. The Examiner's rationale relies on several assumptions that were derived in hindsight, relying on the discoveries and guidance provided in Applicant's specification. Furthermore, Applicant has provided evidence that the claimed methods produce unexpected results that confirm the unobviousness of the invention. For at least the foregoing reasons, Applicant respectfully maintains that the obviousness rejection must be withdrawn.

Applicant asks that all claims be allowed. Enclosed is a $2756 check for the filing fee ($786) and petition for five month extension of time ($1970). Please apply any other charges or credits to Deposit Account No. 06-1050.

Respectfully submitted,

Date: 4/7/03

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